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TITLE: Regional Anesthesia and Valproate Sodium for the Prevention of Chronic Post-Amputation Pain

PRINCIPAL INVESTIGATOR: Thomas E Buchheit MD

CONTRACTING ORGANIZATION: Duke University
Durham NC 27705-4677

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Thomas Buchheit MD
Col. Chester Buckenmaier MD,
Partnering PI Thomas Van de Ven MD, PhD, Investigator
Hung-Lun (John) Hsia MD, Investigator

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Duke University
2200 W Main St Ste 710
Durham, NC 27705

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14. ABSTRACT

The purpose of this research is to determine if FDA approved Valproic Acid, commonly used for migraine headache prophylaxis, will also be effective in the prevention of chronic neuropathic pain. Additionally, this research will define the effect of pre-surgical methylation on the susceptibility to chronic pain, the effect of surgically induced methylation changes on the incidence of chronic pain, and the effect of valproic acid on DNA methylation status.

Because this is a double-blinded, randomized controlled trial, we do not anticipate any major findings until the study is closed and the blinding removed. We are pleased to report that there have been no SAEs attributed to study drug, and that the study drug appears to be well tolerated at all three enrollment sites (Walter Reed National Military Medical Center, Duke, and the Durham VA Medical Center) especially at the Durham VAMC in a generally older, debilitated population.

15. SUBJECT TERMS
Amputation, Postamputation pain, Post-surgical pain, Neuralgia, Epigenetics, Valproic Acid, DNA Methylation, Neuropathic pain

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INTRODUCTION

Chronic pain is a significant problem in patients undergoing surgery following military trauma and chronic vascular disease. Symptoms are typically treated with medications such as narcotics, anti-inflammatory drugs, and local anesthetics. Despite these therapies, more than 60% of patients who have an amputation or significant limb injury experience long-term chronic pain. Chronic pain in military personnel and veterans may impair their ability to ambulate or wear a prosthetic device, and may ultimately require the use of chronic narcotic medications. Although sometimes effective for pain, chronic narcotic medications also carry risks of sedation, confusion, and possible addiction. Identifying preventive mechanisms that can be employed at the time of surgery is of utmost importance for military and veteran health systems. Valproates such as valproic acid have a unique advantage over other classes of medicines used for neuropathic pain, as this drug actually modifies the epigenetic mechanisms, such as DNA methylation, and therefore may demonstrate efficacy in preventing the transition from acute to chronic pain. In this study, we will additionally define the gene expression changes that occur in the transition from acute to chronic pain, and any effect that valproic acid may have on these genes.

In summary, this research will investigate the effectiveness of valproic acid vs placebo when added to regional anesthesia in the prevention chronic pain after amputation, stump revision, or surgery for mangled limb with neurologic damage. It will also define the gene expression changes that occur after surgery and the ability of valproic acid to prevent the epigenetic changes that lead to the development of chronic pain.

KEYWORDS

Amputation, Post-amputation pain, Post-surgical pain, Neuralgia, Epigenetics, Valproic Acid, DNA Methylation, Neuropathic pain

OVERALL PROJECT SUMMARY

We received all approvals necessary to begin enrollment at the Durham VAMC on 22Nov13. As our first year of enrollment (Grant Year 2) saw fewer numbers of eligible subjects because of reduced military conflict, we requested that Duke University Medical Center be added as a third enrollment site. We received approval for enrollment at DUMC on 19May14, from HRPO on 30Jul14, and from DOD on 02 Oct14. With this third study site, we were able to increase enrollment, although still experienced multiple potential study patients excluded by overly rigid inclusion criteria in regards to renal disease (the study drug is hepatically metabolized).

After meetings with the investigational pharmacist and a thorough review of the literature, we removed renal failure from the list of exclusion criteria at Duke University Medical Center on 24 Jun15. An amendment for the same was submitted to the Durham VAMC and approved on 10 Dec 15. This change of inclusion criteria is consistent with the pragmatic "real world" nature of this trial since one of the significant target audiences (chronically ill veterans with vascular disease and diabetes) experiences a high incidence of renal failure. Since the study medication is continued in the treatment of veterans and patients with neuropathic pain, chronic headaches, and bipolar disorder, we believed it appropriate to modify the inclusion/exclusion criteria to mirror standard clinical practice for the treatment of similar conditions.

During year 3 of this research project, we also analyzed our initial VIPER study data, revealing a 65% baseline incidence of chronic post-amputation pain, higher than anticipated at the start of this Valproate grant. The principle investigator has also participated in a series of discussions with other investigators, including those in the IMMPACT Study Group regarding "meaningful" improvements needed to define significance in the setting of a clinical trial. The conclusions of these discussions are also supported by research literature with guidelines now recommending clinical significance to be defined as between 20-30% improvement[1]. With a baseline incidence of 65% chronic pain and a 20% threshold for clinically significant improvement, our statisticians report that 192 total enrolled patients would be required to maintain 80% power for clinical outcomes analysis. Study power expectations were adjusted accordingly during a rebudget process that improved methylation and expression analysis to the latest technology.

In July 2016, our Clinical Research Coordinator (CRC), Veda Byrd, unfortunately left Duke for family reasons. After her departure, we activated separate existing research teams at both Durham VAMC and DUMC. Lani Banez was hired in August to take on the CRC role at DUMC. Research team meetings have been held weekly with Lani and Drs. Buchheit, Van de Ven and Hsia to discuss enrollment goals, and to identify and reduce barriers.

Although enrollment has not yet concluded, our research team has begun collaborative discussions with other Duke investigators regarding comparison and validation cohorts for our anticipated epigenetic findings. The uniqueness of our patient population makes these collaborations attractive, and opens the doors to answer larger questions such as: 1. Are the methylation and expression changes we see in this unique patient population similar to those noted in other chronic pain groups and 2. What are the pathway commonalities (and therefore future therapeutic targets) in these chronic pain syndromes? Such collaborations will be powerful tools in defining the mechanistic universalities involved in the chronification of pain—a question we will be able to address given our analyses at different time points in the injury and subsequent recovery process after amputation.

Below is a detailed list of events and accomplishments during Year 4 of this project.

Durham VAMC

2015

SEPTEMBER	Received approval of the annual Continuing Review from the Durham VAMC on 17 September 2015 with approval through 9 September 2016.
OCTOBER	Submitted Continuing Review approvals to Lori Walther, Human Subjects Protection Scientist. Additionally, an amendment is being prepared for submission to the IRB to relax exclusion criteria to include patients with End Stage Renal Disease.

2016

MARCH	The submitted CR report and supporting documentation accepted by the HRPO
SEPTEMBER	Durham VAMC IRB CR approval sent to the HRPO via email

A total of 40 patients were screened this quarter, 3 were approached and 2 consented. All of the Vascular and Ortho consults were screened, but are not included in those numbers, as most were not amputations. A total of 183 patients were screened for the year of which 7 were consented.

Duke University Medical Center

2015

SEPTEMBER	Amendment submitted to request approval of a phone script for the purposes of conducting pre-screening procedures and obtaining a verbal consent to participate, especially for patients who are admitted over weekends and are first-scheduled surgical cases. Approved on 09/08/2015
OCTOBER	Submitted an updated scientific and budget justifications and an updated budget for Year 4 to Jennifer Shankle, USAMRAA Contract Specialist.
NOVEMBER	Received prior approval for purchase of equipment (specifically a U700 premium 25 cu ft freezer). A revised SOW was submitted to Jennifer Shankle
DECEMBER	A one-time 12 month extension without funds was requested and sent to Jennifer Shankle. The request was endorsed by the Office of Research Administration, Duke School of Medicine.

2016

JANUARY	Fully executed modification of the award received
APRIL	Amendment approved to permit the re-consenting of subjects via U.S. Mail for those not returning to the Duke Clinics for follow-up at the 3-month and 6-month time points whenever a change to the ICF requires re-consenting.

MAY Amendment was approved that requested adding three research team members and removing two research team members.

Amendment was approved to permit scheduling research-related activities (such as submitting orders for the study drug and lab collections) prior to obtaining written consent from the patient.

AUGUST Continuing Review Documents sent to the Human Research Protection Office (HRPO)

September CR documents accepted and approved by the HRPO

A total of 48 patients were screened this quarter, 21 of which were approached for consent and 4 consented and enrolled. Of the scheduled follow-ups this quarter, two one-month follow-ups were completed, five three-month follow-ups were completed, and two six-month follow-ups were completed.

Walter Reed National Military Medical Center

2015

SEPTEMBER Four patients' complete collection of blood samples was shipped to the Duke University Van de Ven lab for analysis

A teleconference was held with Mary McDuffie, Veda Byrd, Dr John Hsia, and Dr Thomas Buchheit. New data collection points and protocol language were discussed.

OCTOBER Received approval from the WRNMMC IRB for an amendment allowing us to capture the presence of wound vac therapy, and permission to administer the PHQ-2 questionnaire to the participants at the 3 and 6 month follow up visits.

NOVEMBER The annual review was approved by the WRNMMC IRB. A new stamped consent was received granting approval thru 12/2016.

2016

JANUARY Approval was received for the revised SOW and the request for a one-time extension without funds.

MARCH The US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) granted approval.

AUGUST An amendment to the CRADA between WRNMMC and Duke University was approved and final signatures obtained on August 15, 2016.

Total follow ups for the year: 13 one month follow ups were done, 12 three month follow ups were done, and 7 six month follow ups.

Important Dates of Multi-Site Study Coordination

2015

SEPTEMBER WRNMMC shipped four patients' complete collection of blood samples to Duke. The samples were logged in our database and are being stored at GSRBI.

OCTOBER Adjudication meetings held on October 9th and 27th for patients meeting 3 month end point analysis. The number of patients with 3 month data was 36 total.

A T-CON was held on 10/16/2015 with Dr Chester Buckenmaier, Dr Thomas Buchheit, Dr Thomas Van de Ven, Veda Byrd, Rachel Morales, Kelly Kiser, Nancy Kwon, Peter Bedocs, and Mary McDuffie. The advantages of whole genome bisulfite methylation sequencing, new analyses, budget plans and considerations, and enrollment expectations were discussed.

NOVEMBER WRNMMC shipped 9 specimens to Duke. The samples were logged in our database and are being stored at GSRBI.

Adjudication meetings held for patients meeting 3 month end point analysis. The number of patients with 3 month data was 43 total.

2016

JANUARY DSMB Report received. Adverse events, protocol deviations and enrollment for all three sites reviewed.

MAY A teleconference was held with Drs. Thomas Buchheit and Thomas Van de Ven, Rachel Morales, Veda Byrd, Col Buckenmaier, Nancy Kwon, Mary McDuffie, and Peter Bedocs. Protocol adjustments, enrollment update, sample shipping, and site visit plans were discussed.

JULY Adjudication meeting held for patients meeting 3 month end point analysis.

SEPTEMBER Adjudication meeting held for patients meeting 3 month end point analysis.

RNA processing begun on pre-study drug samples with good yields noted

OCTOBER Multi-site Study meeting at WRNMMC with investigators at Duke, VAMC, and WRNMMC

- The chart below summarizes enrollment at Durham VAMC, Duke University Medical Center and Walter Reed.

Project Start Date **09/30/2012**

	# Screened	#Enrolled
DVAMC *All approvals received 11/22/2013*		
Year 2, Quarter 1	13	2
Year 2, Quarter 2	16	3
Year 2, Quarter 3	23	4
Year 2, Quarter 4	17	0
Year 3, Quarter 1	28	4
Year 3, Quarter 2	101	0
Year 3, Quarter 3	100	1
Year 3, Quarter 4	47	3
Year 4, Quarter 1	36	1
Year 4, Quarter 2	47	2
Year 4, Quarter 3	60	2
Year 4, Quarter 4	40	2
DUMC *All approvals received 08/25/2014*		
Year 2, Quarter 4	0	0
Year 3, Quarter 1	47	2
Year 3, Quarter 2	39	4
Year 3, Quarter 3	58	4
Year 3, Quarter 4	39	2
Year 4, Quarter 1	53	7
Year 4, Quarter 2	50	3
Year 4, Quarter 3	80	7
Year 4, Quarter 4	48	4
WRNMMC *All approvals received 03/11/2014*		
Year 2, Quarter 2	3	0
Year 2, Quarter 3	26	3
Year 2, Quarter 4	32	4
Year 3, Quarter 1	19	6

Project Start Date **09/30/2012**

	# Screened	#Enrolled
Year 3, Quarter 2	20	7
Year 3, Quarter 3	21	4
Year 3. Quarter 4	19	6
Year 4, Quarter 1	18	3
Year 4, Quarter 2	25	1
Year 4, Quarter 3	21	5
Year 4, Quarter 4	16	3
Total	**1114**	**99**

With the change in both exclusion criteria at Duke (06/24/2015) and VAMC (12/10/2015) and the new screening process by the CRC, we are maximizing opportunities for enrollment during Year 4 of this research.

The SOW dated 18Nov15 is in effect for this year-end report and outlined below.

Task 1 (pre-study) – Human subjects approval (including HRPO) **Months 1-24** *Actual*

a. Duration (Durham VAMC), months 1-9
b. Duration (WRNMMC), months 1-12
c. Exempt Review (Duke), months 9-10 (for blood analysis only)
d. Full Duke IRB Review to add Duke as 3rd enrollment site (August, 2014)

Milestone Pre-Study Task 1a – IRB and HRPO approval in Durham	Month 9	*Month 14*
Milestone Pre-Study Task 1b – IRB approval at WRNMMC	Month 12	*Month 17*
Milestone Pre-Study Task 1c – Duke IRB and HRPO approvals	Month 24	*Month 26*

Task 2 – Clinical Trial **Months 9-57**

Aim 1*: Determine the efficacy of regional anesthesia and valproate in reducing the incidence of chronic post-amputation pain.*

Patients will be screened at the time of scheduling for surgery.
Subjects will receive either placebo or study drug (valproate) TID for 7 days.

a. Subject enrollment at DVAMC (57 pts)	Months 9-57	
b. Subject enrollment at Duke (96 pts)	Months 24-57	
c. Subject enrollment at WRNMMC (70 pts)	Months 12-57	
Milestone Task 2a – First patient enrolled in Durham	Months 9-10	*Month 14*
Milestone Task 2b – First patient enrolled at WRNMMC	Months 12-13	*Month 20*
First enrolled subjects seen at 3 month endpoint	Months 12-16	*Months 17-20*
Milestone Task 2c – First patient enrolled at Duke	Month 24	*Month 26*
Milestone Task 2d – Endpoint adjudication meetings at 6 and 12 mo.	Month 18	*Month 30*
d. Review of site enrollment targets		
Milestone Task 2e – Enrollment of 50% of subjects	Month 45	
e. Interim analysis	Month 45	
Milestone Task 2f – Endpoint adjudication meeting	Months 30	
f. Projected enrollment of 112 subjects complete.	Months 45-46	
Milestone Task 2g – Endpoint adjudication meeting	Month 36	
g. Final subject follow-up after adjudication	Month 48	
Milestone Task – Endpoint adjudication meeting	Month 42	
Milestone Task – Endpoint adjudication meeting	Month 48	

Milestone Task – Endpoint adjudication meeting Month 52

Milestone Task 2h – Close of enrollment for clinical trial Month 57

Task 3 –Epigenetic Genomic and Gene Expression Analysis Months 48-60

Aim 2: Determine the role of differential DNA methylation in post-amputation pain syndromes and their Treatment with valproate.

Identify priority pathways associated with chronic pain phenotypes through DNA methylation sequencing and correlate with gene expression patterns.

a. Determine the effect of pre-surgical methylation status on the incidence of chronic post surgical pain through whole genome bisulfite sequencing. Month 48

-Whole genome bisulfite sequencing of 40 cases and 40 controls before surgery

-Whole genome DNA sequencing of the same 40 cases and 40 controls before surgery

Milestone Task 3a –Initial methylation sequence analysis of 80 patients collected Month 50

Milestone Task 3b – Initial genomic sequence analysis of 80 patients collected Month 50

b. Determine the effect of surgically induced methylation changes on incidence of chronic pain Month 52

-Targeted MethylDIP sequencing of 30 cases and 30 controls before and after surgery.

-RNA sequencing of 30 cases and 30 controls before and after surgery.

Milestone Task 3c – Collect MethylDIP sequence on 120 samples with initial analysis completed Month 54

Milestone Task 3d – Collect RNA seq data on 120 samples with initial analysis completed Month 54

c. Determine the effect of valproic acid on DNA methylation status after surgery Month 54

-Targeted MethylDIP sequencing of 30 placebo and 30 VPA treated patients before and after surgery.

-RNA sequencing of 30 placebo and 30 VPA treated patients before and after surgery.

Milestone Task 3e – Collect MethylDIP sequence on 60 samples with initial analysis completed Month 56

Milestone Task 3f – Collect RNA sequencing data on 60 samples with initial analysis completed Month 56

Milestone Task 3g,3h – Targeted analysis of methylation status at promoter regions of genes of interest with confirmatory gene expression analysis using RT-PCR Month 58

Final Task 3 Milestone – Local investigator meeting for convergence analysis of epigenetic, genomic and RNA expression data Month 60

KEY RESEARCH ACCOMPLISHMENTS

Our research group has recently published granular phenotypic data from our other VIPER research grant[2], demonstrating a 65% incidence of chronic post-amputation pain. This is consistent with historical literature, and higher than our original conservative estimate. We have additionally investigated and accessed improved methods of methylation analysis (whole genome bisulfite methylation sequencing) and targeted methyl-DNA immunoprecipitation sequencing as effective laboratory methods. These two advancements will allow improved outcomes for this research grant.

Our research group has additionally built important collaborative relationships with other Duke laboratories to facilitate the use of comparison/validation cohorts that will allow us to define the mechanistic commonalities in the chronification of pain.

CONCLUSION

Nothing to report.

PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

Kent ML, Hsia H-LJ, Van de Ven TJ, Buchheit TE. Perioperative Pain Management Strategies for Amputation: A Topical Review. Pain medicine (Malden, Mass). The Oxford University Press; 2016 Jul 8; pnw110–6.

Buchheit T. Future therapies and the expanding role for diagnostic ultrasound. Curr Opin Anaesthesiol. 2016 Oct;29(5):582–3

Chamessian A*, Van de Ven TJ*, Buchheit T, Hsia H, McDuffie M, Gamazon ER, Walsh C, Bruehl S, Buckenmaier C, Shaw A. Differential Expression of Systemic Inflammatory Mediators in Amputees with Chronic Residual Limb Pain. Pain. Publish Ahead of Print, 23 September 2016, 10.1097/j.pain.000000000000072. *Co-first authors.

INVENTIONS, PATENTS AND LICENSES

Nothing to report.

REPORTABLE OUTCOMES

Nothing to report.

OTHER ACHIEVEMENTS

Nothing to report.

REFERENCES

1. Dworkin RH, Turk DC, Farrar JT, Haythornthwaite JA, Jensen MP, Katz NP, Kerns RD, Stucki G, Allen RR, Bellamy N, Carr DB, Chandler J, Cowan P, Dionne R, Galer BS, Hertz S, Jadad AR, Kramer LD, Manning DC, Martin S, McCormick CG, McDermott MP, McGrath P, Quessy S, Rappaport BA, Robbins W, Robinson JP, Rothman M, Royal MA, Simon L, Stauffer JW, Stein W, Tollett J, Wernicke J, Witter J. Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. Pain 2005;113:9–19.

2. Buchheit T, Van de Ven T, John Hsia H-L, McDuffie M, MacLeod DB, White W, Chamessian A, Keefe FJ, Buckenmaier CT, Shaw AD. Pain Phenotypes and Associated Clinical Risk Factors Following Traumatic Amputation: Results from Veterans Integrated Pain Evaluation Research (VIPER). Pain medicine (Malden, Mass.) 2015. Epub. Ahead of Print.

APPENDICES

Attachment 1 – Year Four Summary Quad Chart

Regional Anesthesia & Valproate Sodium for the Prevention of Chronic Post-Amputation Pain

Log #PT110575 Award Number W81XWH-12-2-0129

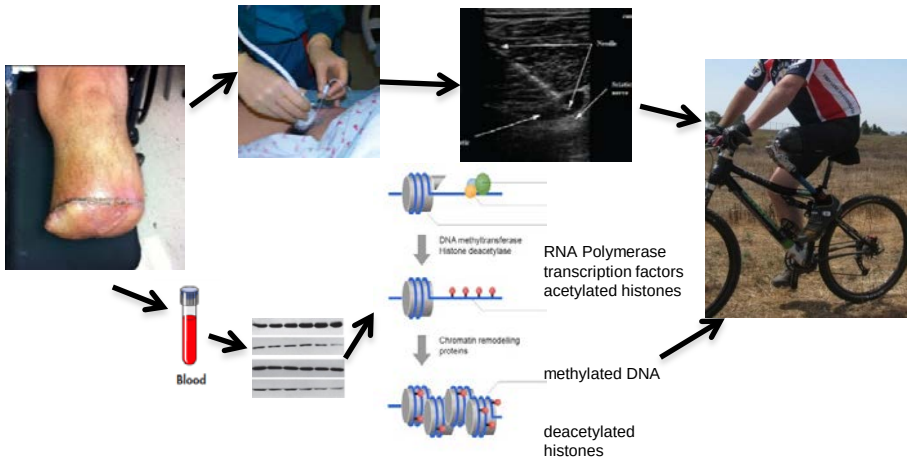
PI: Thomas Buchheit MD **Org:** Duke University **Award Amount:** $2,237,227

Study/Product Aim(s)

- Aim 1: Determine the efficacy of valproic acid combined with regional anesthesia in reducing the incidence of chronic post-amputation pain.
- Aim 2: Determine role of epigenetic DNA methylation in post-amputation pain and effects of valproic acid treatment

Approach

- In a randomized clinical trial, we will determine if the combination of valproic acid combined with regional anesthesia reduces the incidence of chronic post-amputation when compared with regional anesthesia alone.
- We will analyze DNA methylation patterns of patients with post-amputation pain and determine the way they are modified by valproic acid. We will confirm the functional relevance of these modifications using gene expression signatures.



Accomplishments: 1. End-point adjudication of initial 43 patients enrolled 2. Increased study enrollment 3. Improved laboratory epigenetic and genetic analysis techniques

Timeline and Cost

Activities CY	13	14	15	16	17
VA/Duke/HRPO approvals; Durham VA/Duke CRADA approved. Enrollment has begun					
*Enrollment/data collection at VA; *HRPO approval/enrollment at WRNMMC					
Enrollment and data collection, initial analysis					
Enrollment, clinical study closure, outcomes analysis, final adjudication					
Estimated Budget ($K)	**$296K**	**$398K**	**$459K**	**$542K**	**$542K**

Updated: October 12, 2016

Goals/Milestones

CY13 Goal – Protocol planning, data use agreements, IRB & HRPO approvals, lab supply purchasing and enrollment

☑ Fully planned, IRB approval at Duke & VAMC, lab supplies purchased and lab analyses developed. CRADA between VA & Duke approved.

CY14 Goals – Patient enrollment, data and sample collection

☑ 1st patient enrolled 12/13 at Durham VAMC

☑ IRB approval & HRPO secondary approvals, Duke Enrollment

CY15 Goal – Patient enrollment, data collection, clinical adjudication

☑ Increased enrollment with 3rd study site, endpoint adjudications

CY16 Goal – Clinical study closure and outcomes analysis

☐ Additional enrollment and endpoint adjudication

☐ Clinical outcomes analysis

CY17 EWOF Goal – Additional enrollment

☐ Final epigenetic analysis and endpoint adjudication

Comments/Challenges/Issues/Concerns

- Enrollment continues at all 3 sites
- RNA extraction for expression analysis has begun

Budget Expenditure to Date (from start to date)

Projected Expenditure: $1,668K

Actual Expenditure: $1,573K